ADAPTATION TO SALT OF THE PHOTOSYNTHETIC APPARATUS IN CYANOBACTERIA

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1. Introduction

Cyanobacteria grow under a wide range of environmental conditions. Their distribution in environments of highly diversified osmotica is well documented [1]. Hence, the photosynthetic apparatus must adapt to the different degrees of salinity in the habitat. This is achieved by means of either osmoregulation or on the level of the organism [2] or modification of the enzyme structure [3].

Here we correlated the salt tolerance of the whole organism with the salt tolerance of activities of the photosynthetic apparatus and NADPH-cytochrome *c* reductase in 4 cyanobacteria characterized by different degrees of salt tolerance: marine *Spirulina* tolerates up to 3-times sea water [4]; *Calothrix scopulorum*, sea water [5]; *Spirulina platensis*, brackish water [6]; *Nostoc muscorum* 7119, fresh water.

We show that the soluble components of the final steps of photosynthetic electron transport constitute a salt-sensitive site and that salt tolerance may be acquired during growth in saline water.

2. Materials and methods

2.1. *Organisms and growth conditions*

Spirulina platensis (Cambridge culture collection no. 1475/4a) was grown in Zarouk's medium [8] in a turbidostat culture at 35°C. The culture was stirred by an air stream containing 1.5% CO_2 at 35°C and illuminated by cool white light at 1250 $\mu W.cm^{-2}$. Marine *Spirulina* obtained from Dr A. Abeliovich, was grown in batch cultures in sea water containing (g/l): $NaNO_3$, 2.5; $NaHCO_3$, 2; $FeSO_4$, 0.01; $Na_2 \cdot EDTA$, 0.08; Na_2HPO_4, 0.5; and 1 ml of A_5 and B_6 microelements solution [4]. The cultures were grown on a rotary shaker at 30°C and illuminated by cool white light at 250 $\mu W.cm^{-2}$. *Nostoc muscorum* 7119 [9] and *Calothrix scopulorum* (Cambridge culture collection no. 1410/5) were grown in the medium of [10] in batch cultures on a rotary shaker at 30°C and illuminated at 250 $\mu W.cm^{-2}$.

2.2. *Cell-free preparations*

Algae were harvested at late logarithmic phase, washed and resuspended in 25 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethane-sulphonate (Hepes) (pH 7.5) disrupted by sonication and centrifuged (27 000 × *g* for 30 min). The supernatant was assayed for reduction of cytochrome *c* and the pellet, resuspended in Hepes buffer, was used for the assay of the photoreduction of methylviologen mediated by photosystem 1.

Protein was determined according to [11], and chlorophyll extracted in 80% acetone was determined as in [12].

2.3. *Assays*

Oxygen evolution was measured in cells, which had been washed and resuspended in fresh medium at 30°C with a Clark-type electrode (YSI 4004, Yellow Springs Instr., OH) connected to a recorder. Cells were illuminated at I=3.5 $mW.cm^{-2}$. Methylviologen photoreduction was measured as O_2 consumption with an oxygen electrode. Reduction of cytochrome *c* (horse heart, Sigma) was measured at 550 nm in a Gilford 250 spectrophotometer.

3. Results and discussion

3.1. *Effect of salt on photosynthetic electron transport*

The response to salt of photosynthetic electron transport in vivo was tested by following O_2 evolution in intact filaments of 4 different cyanobacteria in their growth medium (fig.1). The photosynthetic activity of marine *Spirulina* tolerated high salt concentrations with no inhibition up to 2 M NaCl, while *Calothrix* was moderately sensitive and *Nostoc* and *S. platensis* were much more sensitive to NaCl. The photoreduction of methylviologen, mediated by photosystem 1 and supported by reduced dichlorophenolindophenol, was tolerant to NaCl when tested in membrane preparations of *Nostoc, Calothrix* and *S. platensis* as shown in fig.2. These results suggest that the membrane-bound components which participate in the photosystem 1 reaction of electron transport are insensitive to NaCl.

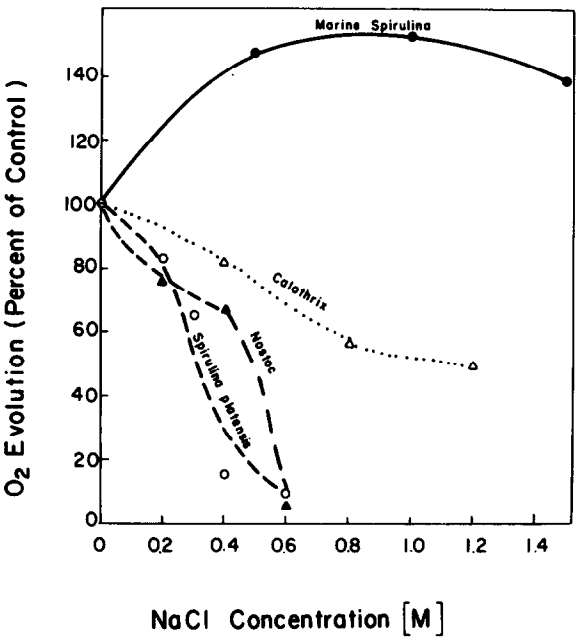

Fig.1. Effect of salt on O_2 evolution. Cultures grown as in section 2 were harvested at late logarithmic phase of growth. Cells containing 3–10 μg chl were resuspended in growth medium except for marine *Spirulina* which was resuspended in artificial sea water without NaCl. 100% activity: 82, 206, 138 and 271 μmol O_2 evolved mg . chl^{-1}. h^{-1} for marine *Spirulina, Calothrix, Nostoc* and *Spirulina platensis*, respectively.

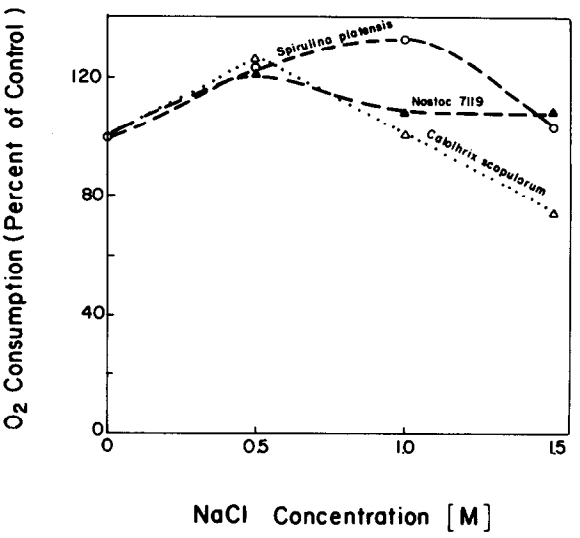

Fig.2. Effect of salt on the activity of photosystem 1. Reaction mixture (4 ml) contained 50 mM Hepes (pH 7.5), 5 mM sodium ascorbate, 50 μM dichlorophenolindophenol, 50 μM methylviologen, 2.5 mM sodium azide and membranes containing 20–50 μg chl. 100% activity: 730, 520 and 380 μmol O_2 consumed . mg chl^{-1}. h^{-1} for *Spirulina platensis, Nostoc* 7119 and *Calothrix scopulorum*, respectively.

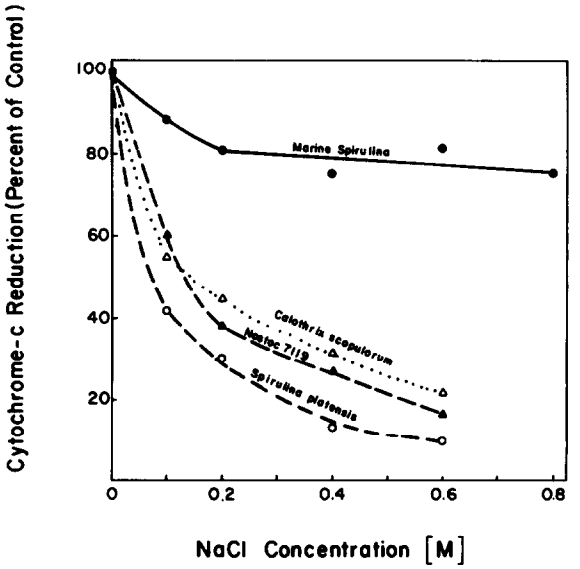

Fig.3. Effect of salt on NADPH–cytochrome *c* reduction. Activity was measured in a 1 ml reaction mixture containing 25 mM Hepes (pH 7.5), 5 mM $MgCl_2$, 33 μM NADPH, 33 μM cytochrome *c* and sonicated supernatant cells containing 1–2 mg protein. 100% activity: 3.6, 10.2, 5.0 and 5.5 μmol cytochrome *c* reduced . mg $protein^{-1}$. min^{-1} for marine *Spirulina, Calothrix scopulorum, Nostoc* 7119 and *Spirulina platensis*, respectively.

3.2. *The effect of salt on the activity of NADPH-cytochrome c reductase*

The effect of salt on the reduction of cytochrome *c* by NADPH was tested in the crude soluble fraction of ruptured cells of the 4 different cyanobacteria. As shown in fig.3, only the preparation derived from the marine *Spirulina* exhibited tolerance to NaCl. The reduction of cytochrome *c* involves the formation of a complex between ferredoxin and ferredoxin-NADP reductase [13], which was found to be sensitive to high ionic strength in higher plants [14]. The difference in salt sensitivity between marine *Spirulina* on the one hand and *Nostoc, Calothrix* and *S. platensis* on the other, suggests that the enzyme couple in the marine *Spirulina* has become modified in a way which allows the formation of a stable active complex at high ionic strength. The soluble components participating in the final steps of electron transport are therefore a salt-sensitive site.

3.3. *Acquired tolerance to salt*

We have tested the possibility that cyanobacteria may acquire salt tolerance during their growth in media containing salt. *S. platensis* was cultured in growth media containing either 0.1 M or 0.2 M NaCl.

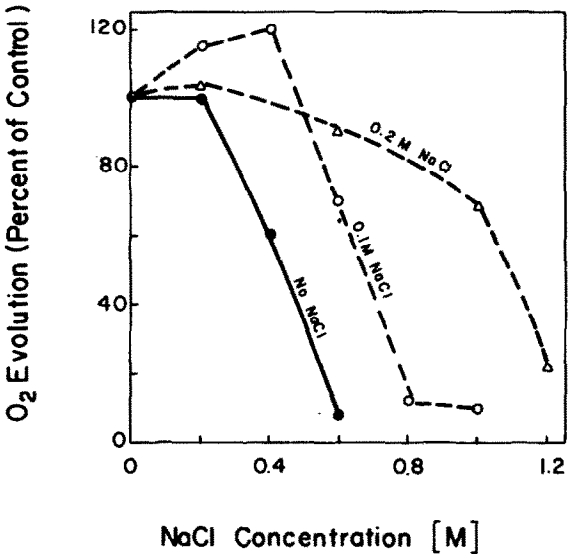

Fig.4. Effect of salt on O_2 evolution in cultures of *S. platensis* previously grown at different salt concentrations. Cells grown at 0, 0.1 and 0.2 M NaCl in turbidostat cultures at identical conditions were harvested, washed and resuspended in salt-free medium. 100% activity: 469, 450 and 413 μmol O_2 evolved . mg chl^{-1}. h^{-1} for cultures containing 0, 0.1 and 0.2 M NaCl, respectively.

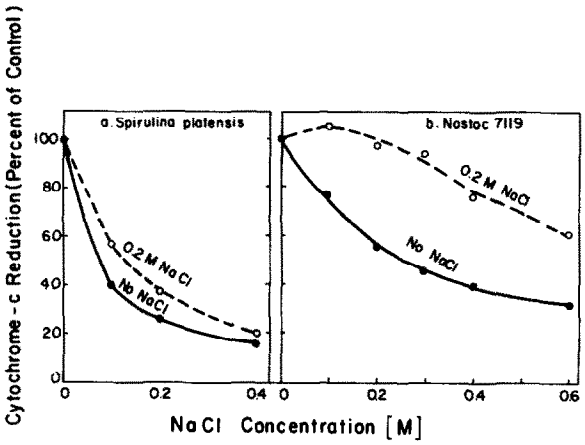

Fig.5. Effect of salt on NADPH–cytochrome *c* reduction in extracts from cultures previously grown at different salt concentrations. Cells were harvested, washed, disrupted in 25 mM Hepes and the supernatant was assayed as in fig.3. 100% activity: (a) 5.5 and 7.6 μmol cytochrome *c* reduced . mg $protein^{-1}$. min^{-1} for *Spirulina* grown with 0 and 0.2 M NaCl, respectively. (b) 5 and 8.5 μmol cytochrome *c* reduced . mg $protein^{-1}$. min^{-1} for *Nostoc* grown with 0 and 0.2 M NaCl, respectively.

The photosynthetic activity of these cultures exhibited increased tolerance to salt (fig.4). Some acquired tolerance to salt was also found in the activity of NADPH-cytochrome *c* reductase (fig.5a).

Similar trends were also found in cultures of *Nostoc* grown in batch cultures containing either 0.2 M or 0.4 M NaCl. The photosynthetic activity was found to tolerate higher salt concentrations than the control cultures, and likewise the reduction of cytochrome *c* exhibited adaptation to salt (fig.5b). These results suggest that the enzyme couple ferredoxin and ferredoxin-NADP reductase undergoes a specific modification, which confers on it an increased tolerance to increased ionic strength. The reports that ferredoxin-NADP reductase is composed of a mixture of isozymes [13] and the evidence for two ferredoxins in *Nostoc* [15] may provide a possible explanation for this modification during the growth in salt-containing medium; in this medium, the cells may preferably synthesize the isozyme which is more resistant to the high ionic strength.

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